Borrower's Name	Alex Noguerola	Org. or A.U.	1744	Phone	305-5686
Serial Number	08/845,660	Date of Request	6/11/99	Date Needed By	6/16/99

Please Attach Copy of Abstract, Citation, Or Bibliography. If Available Please Provide Complete Citation. Only One Request Per Form.

Author/Editor:	See attached sheet. Date of Publication
Journal/Book Title:	
Article Title:	
Volume (Issue):	
Pages:	
Year of Publication:	
Publisher:	
Remarks:	COMPLETED

BEST AVAILABLE COPY

STIC Use Only

Accession Number: 253[redacted]610

LIBRARY ACTION	LC		NAL		NIH		NLM		NBS		PTO		OTHER	
	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
Local Attempts														
Date														
Initials														
Results														
Examnr. Called														
Page Count														
Money Spent														

Provided By: Source and Date

Ordered From: Source and Date

Remarks/Comments
- 1st & 2nd denotes times taken to a library
- FX - Means Faxed to us
- O/N - Under NLM=Overnight Service

L2 ANSWER 12 OF 45 CAPLUS COPYRIGHT 1999 ACS
AN 1996:286047 CAPLUS
DN 125:51710
TI Construction of porphyrin- and flavin-conjugated .alpha.-**helix peptides**
AU Mihara, Hisakazu; Sakamoto, Seiji; Haruta, Yasuaki; Aoyagi, Haruhiko
CS Tokyo Institute Technology, Faculty Bioscience and Biotechnology, Yokohama, 226, Japan
SO Pept. Chem. (1996), Volume Date 1995, 33rd, 517-520
CODEN: PECHDP; ISSN: 0388-3698
DT Journal
LA English
CC 6-3 (General Biochemistry)
AB Amphiphilic .alpha.-**helix peptides** carrying porphyrin or flavin moieties were designed and synthesized. The tetraphenylporphyrin and 7-acetyl-10-methylisoalloxazine were able to be fixed and arranged with a chiral twist in a 3D structure constructed by the amphiphilic folding of .alpha.-**helixes**. Furthermore, assembly of the **peptides** on a gold **electrode** was examd.
ST porphyrin flavin alpha **helix** peptide prepn
IT Flavins
Porphyrins
RL: PRP (Properties)
(construction of porphyrin- and flavin-conjugated .alpha.-**helix peptides**)
IT Conformation and Conformers
(.alpha.-**helical**, construction of porphyrin- and flavin-conjugated .alpha.-**helix peptides**)
IT 174546-26-0P 178056-35-4P 178095-57-3P
RL: PRP (Properties); SPN (Synthetic preparation); PREP (Preparation)
(construction of porphyrin- and flavin-conjugated .alpha.-**helix peptides**)
IT 61449-63-6 68973-54-6
RL: RCT (Reactant)
(construction of porphyrin- and flavin-conjugated .alpha.-**helix**

Peptide Chemistry 1995: N.Nishi (Ed.)
Protein Research Foundation, Osaka (1996)

Construction of Porphyrin- and Flavin-Conjugated α-Helix Peptides

Hisakazu Mihara[a], Seiji Sakamoto[b], Yasuaki Haruta[b] and Haruhiko Aoyagi[b]

[a]*Department of Bioengineering, Faculty of Bioscience and Biotechnology, Tokyo Institute of Technology, Midori-ku, Yokohama 226, Japan*
[b]*Department of Applied Chemistry, Faculty of Engineering, Nagasaki University, Bunkyo-machi, Nagasaki 852, Japan*

Amphiphilic α-helix peptides carrying porphyrin or flavin moieties were designed and synthesized. The tetraphenylporphyrin and 7-acetyl-10-methylisoalloxazine were able to be fixed and arranged with a chiral twist in a 3D structure constructed by the amphiphilic folding of α-helices. Furthermore, assembly of the peptides on a gold electrode was examined.

Introduction

Studies on natural proteins applicable for electronic devices, for example, bacteriorhodopsin and bacterial photosynthetic reaction center, have been accumulated at the level of atomic resolution. Along with these findings, much attention has been focused on construction of 3D structures of polypeptides containing functional chromophores. Considerable efforts have been devoted to the *de novo* design of polypeptides and proteins with stabilized and pre-defined structures [1]. Ultimate goal of the protein design would be to know the principles in highly efficient functions of natural proteins and to produce artificial molecules with such functions. We have investigated the designed peptides which have a protein-like tertiary structure composed of α-helices [2]. On the basis of these studies, we have found that the designed peptides could organize orientation of functional chromophores such as porphyrin, pyrene, Ru-trisbipyridine, and other photo- and redox-active groups, on the α-helical scaffold. In this study, we investigated chiral assembly of tetraphenylporphyrin and flavin which were anchored on side chains of newly designed amphiphilic α-helix peptides (Fig. 1). Dependence of the porphyrin- and flavin-assembly on the conformational changes of the peptides was examined. Furthermore, orientation of the peptides in monolayer on a gold surface was attempted for the future design of peptidyl devices.

Results and Discussion

A 14-peptide was designed to take an amphiphilic α-helix structure, in which a hydrophobic amino acid Leu, charged amino acids Lys and Glu, and non-charged hydrophilic amino acids Ala and Gln were deployed on the helix in a manner similar to coiled-coil proteins (Fig. 1). 5-(4-Carboxyphenyl)-10,15,20-tris(4-methylphenyl)-porphyrin (Por) was introduced on the side chain of Lys at the 6th position instead of Leu (Por1α). 7-Acetyl-10-methylisoalloxazine (Fla) was introduced on the Cys side

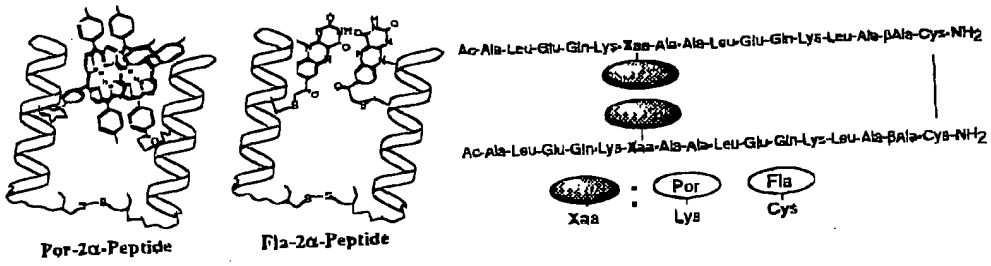

Fig. 1. Structure of Por- and Fla-2α-Peptides.

chains at 6, 7 and 8th positions (Fla1α). The Por- and Fla-peptides were dimerized by the disulfide linkage of Cys residues (Por2α and Fla2α). The peptides were synthesized by SPS method using Fmoc chemistry [Lys(Boc)[6], Lys(CIZ)[5,12] for Por-peptides, Cys(Trt)[6,7,8] for Fla-peptides, Cys(Acm)[16], and other protecting groups were of Fmoc-strategy]. The *N*-hydroxysuccinimide ester of the porphyrin was reacted with the Lys[6] side chain of the partially-protected (CIZ) peptides. The CIZ groups of the Por-introduced peptides were deprotected by TMSOTf [3]. 7-Bromoacetyl-10-methylisoalloxazine was reacted with Cys side chains. The Acm group was removed with $AgBF_4$ [4] and the dimeric peptides were obtained by air oxidation. FAB-MS and SDS-PAGE confirmed the structures of 1α and 2α peptides, respectively. Gel-filtration of Por1α and Por2α suggested that both peptides were in tetrameric and dimeric form, respectively, to take a 4α-helix 3D structure in aqueous solution.

CD spectra at the amide region showed that the Por-peptides took highly α-helical structures (Fig. 2). The α-helicity $[\theta]_{222}$ of Por1α in a buffer solution was slightly larger than that of Por2α. The 1α-peptide without the porphyrin could not take an α-helical structure in aqueous solution. Therefore, the stabilization of the α-helix structure of Por1α is ascribed to the peptide assembly by the large and hydrophobic porphyrin. It appears that Por1α is flexibly assembled to a 4α-helix conformation with higher α-helicity than Por2α. At the Soret band of the porphyrin, great CD spectra were observed in aqueous solution, which were shown as split shape ($\Delta[\theta]_{430\text{-}405} = 4.0 \times 10^6$ deg cm^2 dmol^{-1} for Por1α and 2.0×10^6 for Por2α). The great chiral twist between the porphyrins indicates that the porphyrin moieties are highly oriented in close positions in the four-helix bundle structure [5]. The absorption spectra of the porphyrins at the Soret band were split at 406 (B_y) and 423 nm (B_x), and both Soret and Q bands were in extremely reduced intensity and red-shifted compared with those in TFE. The fluorescence intensities of the peptides were also reduced in ca. 1/10 compared with those in TFE.

Because the amphiphilic folding of peptides could be regulated by the addition of organic solvent, the spectra were measured at the various content of TFE (Fig. 3). At the first stage of the addition (<40%), the ellipticity at 430 nm were increased with increasing TFE content, and reached to a maximum at 30% (Por1α) and 40% (Por2α) TFE. At the contents, the intensities were enhanced in 4-6 fold ($\Delta[\theta]_{430\text{-}405} = >1 \times 10^7$ deg cm^2 dmol^{-1}). With further addition of TFE, the split CD disappeared at 70-80% TFE. The α-helicity $[\theta]_{222}$ behaved similar to the split CD. The diminished intensities in the absorption and the fluorescence spectra were canceled by the TFE addition. In TFE, the spectra were similar to those of the porphyrin. The TFE-titration experiments revealed that the chirally twisted orientation of the porphyrins reached to a maximum when the peptide 3D structures were untied to some extent. In higher TFE contents, the porphyrins at side chains are free to move, resulting in complete loss of the chiral orientation. The bundle conformation of Por2α was destroyed by the higher TFE content (40%) than Por1α was (30%), probably due to the covalent bonding of the 2α-peptide.

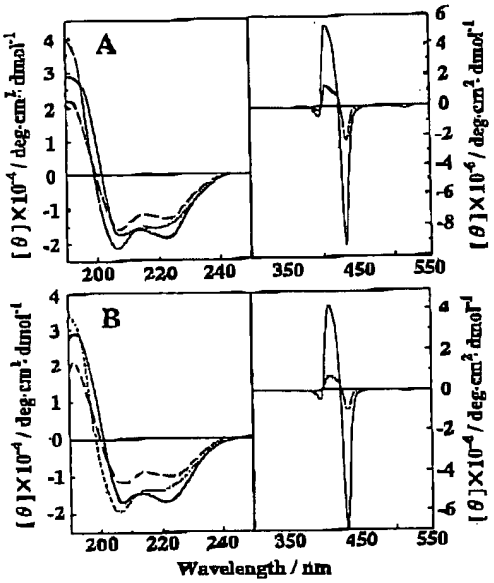

Fig. 2. CD spectra of Por1α (A) and Por2α (B) in 20 mM TrisHCl (pH 7.4) (– – –), 40% TFE/buffer (———), and TFE (- - - -). [θ] at amide and porphyrin regions are indicated per residue and porphyrin, respectively. [Por] = 20 μM, 25 ℃.

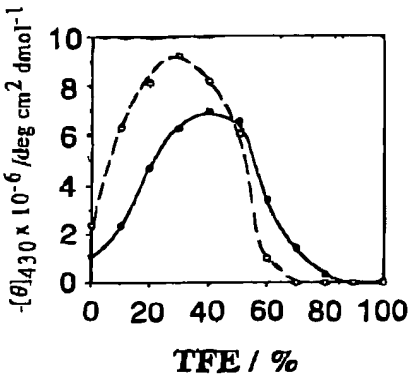

Fig. 3. Dependence of the CD spectra at 430 nm on TFE content. (- - - -) Por1α and (———) Por2α.

CD spectra of Fla-peptides were measured in buffer and TFE (Fig. 4). Fla1α did not take an α-helix structure in aqueous solution, while Fla2α folded in an 2α-helix structure in buffer. However, the α-helicities were not high, especially for Fla^{6}2α. The rather hydrophilic group, Fla, seems to prevent the amphiphilic folding of the 2α-helix peptides. Both 1α and 2α peptides attained α-helix structures in TFE. The flavin CD at the side chains were observed and changed dramatically depending on circumstances and the conformation. These results suggested that the Fla groups were chirally oriented on the α-helical scaffold and the orientation was regulated by the peptide conformation. The absorption and fluorescence spectra confirmed the CD results.

The Por(Mn)- and Fla-peptides were adsorbed on a gold surface of an electrode through the sulfide linkage. The cyclic voltammetry revealed that the functional groups, Por(Mn) and Fla, were redox active on the electrode, and the peptides bound on the surface as an α-helical monolayer (Fig. 5). Furthermore, the Fla-peptides mediated the electron transfer from the electrode to $[Fe(CN)_6]^{3-}$ and cytochrome *c* in a vector manner.

The chiral assembling of porphyrins and flavins was achieved by the combination of the designed α-helical peptides. The defined spatial orientation of functional groups by polypeptide 3D structures could be applied to elucidation of roles of polypeptide structures on proteinous functions, and obtained information will be useful to design artificial proteins. The successful assembly of the peptides with pre-defined conformation on a surface will be useful for evaluating characters of artificial peptides and further developing peptidyl devices.

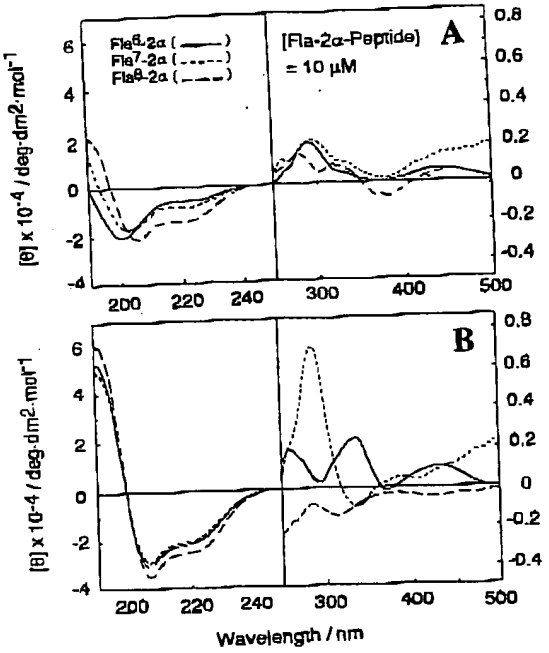

Fig. 4. CD spectra of Fla2α-peptides in buffer (A) and TFE (B). (——) Fla^6, (- - - -) Fla^7, and (— — —) Fla^8. [Fla] = 10 μM

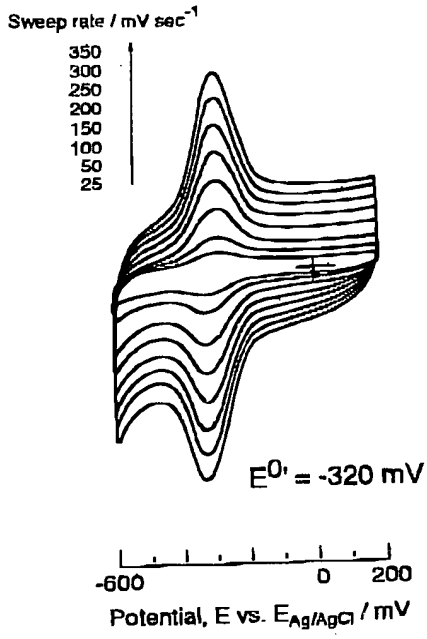

Fig. 5. Cyclic voltammogram of Fla^8]α in 100 mM phosphate (pH 7.0).

Acknowledgements

We thank Professor Norikazu Nishino, Kyushu Institute of Technology, for the generous gifts of porphyrin and flavin. This work was supported in part by Grant-in-Aids for Science Research from the Ministry of Education, Science, Sports and Culture.

References

1. S. F. Betz, D. P. Raleigh and W. F. DeGrado, *Curr. Opin. Struct. Biol.*, **3**, 601-610 (1993).
2. H. Mihara, Y. Tanaka, T. Fujimoto and N. Nishino, *J. Chem. Soc., Perkin Trans. 2*, **1995**, 1133-1140; H. Mihara, Y. Tanaka, T. Fujimoto and N. Nishino, *J. Chem. Soc., Perkin Trans. 2*, **1995**, in press and references cited therein.
3. N. Fujii, A. Otaka, O. Ikemura, K. Akaji, S. Funakoshi, Y. Hayashi, Y. Kuroda and H. Yajima, *J. Chem. Soc., Chem. Commun.*, **1987**, 274-275.
4. M. Yoshida, T. Tatsumi, Y. Fujiwara, S. Iinuma, T. Kimura, K. Akaji and Y. Kiso, *Chem. Pharm. Bull.*, **38**, 1551-1557 (1990).
5. S. Matile, N. Berova, K. Nakanishi, S. Novkova, I. Philipova and B. Blagoev, *J. Am. Chem. Soc.*, 117, 7021-7022 (1995).